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# GENETIC FEATURES OF *CHORISTONEURA OCCIDENTALIS*, *C. VIRIDIS*, AND *C. FUMIFERANA* POPULATIONS EXHIBITING DIFFERENTIAL RESPONSE TO INSECTICIDES


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1. SUMMARY

Significant differences in toxicological response to acephate occurred among the spruce budworm groups examined. From least susceptible to most susceptible, the order was: C. occidentalis laboratory colony, C. viridis laboratory colony, C. fumiferana laboratory colony, and C. occidentalis McCall field population. The relative differences in response (comparison of $LD_{50}$ values) between laboratory populations of C. occidentalis and C. viridis were less than 1.25X in both 1977 and 1978 tests; however, when the responses of these species to carbaryl were compared, C. viridis was 4 times more tolerant than C. occidentalis.

In overall genetic relationship, the laboratory and McCall populations of C. occidentalis were most alike and C. viridis and C. fumiferana were most dissimilar. Details of genetic variation at certain esterase gene loci in C. occidentalis appear correlated with response to acephate. The genetic basis for differential insecticide response within species was substantiated by the large differences in response which occurred among sibling groups of C. occidentalis and C. viridis. Genetic comparison of untreated larvae and survivors of insecticide treatment from several sibling groups suggested that C. occidentalis larvae carrying allozyme #3 at EST2 had a higher survival rate and could be characterized as most tolerant to acephate.

The responses of C. occidentalis instars 2-6 to acephate (Orthene 75S) did not differ significantly. However, responses of these developmental stages to carbaryl (Sevin-4-oil) were neither equal nor parallel. Instars 4 and 5 were most tolerant of carbaryl. Shifts in gene expression over larvae development are being examined in relation to these differences in susceptibility.

## 2. INTRODUCTION

Host tree preferences and other important characteristics differ markedly among spruce budworm species (Freeman 1967, Freeman and Stehr 1967, Sanders 1974). Recognizing and evaluating such diversity within the Choristoneura complex is essential for development of realistic and effective pest management strategies (Clark et al. 1967, Wellington 1977). Only when variation within a group is recognized and assessed can studies based on one population or species be extrapolated accurately to other untested species. Forexample, if no insecticides have been tested on a particular species, it has been accepted practice to estimate the response of an untested species from results of tests on other species of the same genus. It is of practical value to know the extent of variation in response of the various spruce budworm species to insecticides being developed for forest insect control and to provide an estimate of the practicality of such extrapolation within the genus. As a result, Robertson et al. (1978) compared the response of 6 Choristoneura species to selected carbamate, chlorinated hydrocarbon, organophosphorus, and pyrethroid insecticides. They found that no single species was consistently the most or least susceptible to those chemicals at $LD_{50}$.

Even within a single species, larval stages may vary in their response to the same chemical. An investigation of spray and feeding toxicities of acephate and carbaryl to instars 1-6 of the western spruce budworm (C. occidentalis) revealed small differences in overall relative susceptibility to acephate and substantial differences in susceptibility to carbaryl (Robertson, in prep.).

These results between and within Choristoneura species indicate that extrapolating the response of one spruce budworm group to another should be avoided. Unequal response to insecticides shown by these studies could significantly and adversely affect the results of control operations based on such extrapolations. Such work underlines the necessity for additional research into the diversity of toxicological response of Choristoneura spp. before insecticides are applied against them in large-scale control programs.

Because they provide a means of identifying resistant genotypes, electrophoretic techniques have been of great value in relating specific enzymes to insecticide response (Georghiou and Pasteur 1978). Several studies have demonstrated that changes in esterase sensitivity to inhibition by organophosphate and carbamate insecticides can confer high levels of resistance (Plapp 1976). Marked differences occur in esterase zymograms of susceptible and resistant groups (e.g., Sudderuddin 1973, Beranek 1974, Pasteur and Sinegre 1975, Stock and Robertson 1979). Electrophoretic differences have also been reported among strains of insects resistant to pyrethroid insecticides (Sawicki and Devonshire 1979).

Our purpose was to examine in more detail both inter- and intraspecific variation in toxicological response in selected Choristoneura species. We sought to define the underlying genetic basis for any observed differences in response and to identify genetic markers which might aid in predicting the response of spruce budworm species to insecticides before the insecticides are applied in a control program. Specifically, our objectives were the following:

1) Species Comparison

   a) Compare toxicological responses of C. occidentalis, viridis, and fumiferana to acephate (Orthene$^{R}$, an organophosphate);

compare responses of C. occidentalis and viridis to carbaryl (Sevin$^{R}$, a carbamate).

b) Define overall genetic relationships among C. occidentalis, viridis, and fumiferana.

c) Identify correlations, if any, between toxicological response and genetic composition.

2) Intraspecific Variation

a) Compare sibling group responses for single populations of C. occidentalis and viridis; compare genetic makeup of control and survivor groups.

b) Compare toxicological response and identify genetic relationship between laboratory and field populations of C. occidentalis.

c) Compare toxicological response of different larval stages of C. occidentalis to acephate and carbaryl; look for differences in esterase expression that might be associated with alterations in response during development.

## 3. METHODS

### Bioassay

Last-instar C. occidentalis and C. viridis, reared by the methods described by Robertson (1979), were obtained from non-diapausing laboratory colonies at Berkeley. Diapausing C. fumiferana were obtained from the Pest Management Institute, S. Ste. Marie, Canada, and were reared to the last stage. For tests of sibling group responses with C. occidentalis and viridis, separate rearing containers were used for individual egg masses gathered from single-pair matings; larvae were therefore separated by sibling group.

Sixth instars 60-100 mg in weight were then selected for testing. In addition, larvae from an epidemic population of C. occidentalis from McCall, Idaho, were obtained, reared to the 6th instar, and tested. For tests of instar response, instars 2-6 were selected from the C. occidentalis laboratory colony.

Procedures for formulation and topical application are described by Robertson et al. (1976). Sprays of Orthene 75S (acephate) and Sevin-4-Oil (carbaryl) were applied as described by Robertson and Boelter (1979). Five concentrations of each chemical were tested in each of 3 replicates. Thirty insects were tested with each concentration in each replicate. For each replication of sibling group tests, 3-15 sibling groups were tested with each concentration and acetone alone. In the complete test of each chemical, 275-781 insects, representing 13-15 sibling groups, were treated with insecticide and 118-201, representing the same sibling groups, were left untreated. Mortality counts were taken 7 days post-treatment and survivors frozen for genetic analysis. Data from mortality counts were analyzed by probit analysis (Russell et al. 1977), and dose-response lines were statistically compared by likelihood ratio tests (Savin et al. 1977).

Genetic Studies

Survivors of treatment and control larvae were subjected to biochemical genetic analysis using starch gel electrophoresis. Insects were ground in 2-5 drops of distilled water, the homogenate centrifuged and the supernatent absorbed onto small paper wicks. Wicks were inserted in a gel prepared from a 13% solution of hydrolyzed potato starch and buffer. The buffer system used for electrophoresis of budworm esterases was described by Ridgway et al. (1970). In preparing this buffer, a stock solution (0103 M tris-hydroxymethylaminoethane and 0.005 M citric acid) and an electrode buffer (0.06 M

lithium hydroxide and 0.3 M boric acid) were made. A 99:1 mixture of stock solution and electrode buffer was used in the gels. Electrode buffer was used undiluted in the trays.

Electrophoresis was carried out at 75 mA until a dye marker moved 4-6 cm from the origin in the anodal direction. Gels were then sliced and both the anodal and cathodal slices stained for specific enzyme types. The stain used for budworm esterases is described by Bush and Huettel (1972) and contains both alpha- and beta-naphthylacetate substrates.

The genetic makeup of each group of larvae was recorded as genotype and allele frequencies for each enzyme-producing gene locus. The method of Krimbas and Tsakas (1971) was used to calculate frequencies of the silent allele at EST2. Contingency chi-square tests were used to seek differences at individual loci among populations. Rogers' (1972) index of genetic similarity was calculated between populations over all loci and over esterase loci alone to determine levels of genetic similarity among untreated laboratory and field populations of spruce budworm larvae.

## 4. RESULTS

### 1) Species Comparison

a) Toxicological comparisons. The responses of C. occidentalis, viridis, and fumiferana to acephate (Table 1, A) were neither parallel (-2 log L=9.55;d.f.=3;p=0.023) nor equal (-2 log L=78.1;d.f.=6;$p<0.001$) in 1977 experiments. The decreasing order of susceptibility, evaluated by ranking the $LD_{50}$s, was: C. occidentalis (lab), C. viridis, C. fumiferana, and C. occidentalis (McCall). In the 1977 experiments, the responses of C. occidentalis and C. viridis were parallel (-2 log L

=1.39;d.f.=1;p=0.237) but not equal (-2 log L=6.02;d.f.=2;p=0.049). When these two species were compared again in 1978 (Table 1, B), their responses were both parallel (-2 log L=3.39;d.f.=1;p=0.065) and equal (-2 log L=4.29;d.f.=2;p=0.117).

Responses of C. occidentalis and viridis to carbaryl (Table 2) were parallel but not equal. The probability of the hypothesis of equality was less than 0.001 (-2 log L=31.6;d.f.=2), while that of the hypothesis of parallelism was 0.192 (-2 log L=1.70;d.f.=1). Differences in species responses noted in these experiments confirm previous observations by Robertson et al. (1978).

b) Genetic relationships. Isozymes produced by 17 separate gene loci were examined in C. occidentalis, viridis, and fumiferana (Tables 3, 4). At least 5 polymorphic gene loci code for spruce budworm esterases (Table 5, Fig. 1). Overlap of the slow allozyme of EST2 and the fast allozyme at EST3 was resolved by the differential preference of the two loci for the naphthylacetate substrates. All loci except EST2 exhibit a preference for alpha-naphthylacetate and stain a dark blue color. EST2 allozymes stain red. Data were obtained on 15 loci representing 9 enzyme types (Table 6). Eight of these loci (EST2,3,5; AAT1;LAP1,2,3; PGM) exhibited polymorphism in one or more of the populations. Seven (AAT2, EST6, LDH, ME, TFN, AcP, TO) were monomorphic in all larvae examined for this study.

Major differences between groups (Table 6) occurred at EST2, where the common allozyme was different in C. viridis from all other groups, and at AAT1, where all groups except C. fumiferana were fixed for the slow (#3) allozyme. Rogers' similarity coefficients, cal-

culated between groups over all loci (Table 7, unbracketed numbers), showed that the highest similarity (.97) occurred between the laboratory and field populations of C. occidentalis and that the lowest overall similarity (.77) occurred between the C. viridis and the C. fumiferana laboratory colonies. Based on the esterases alone (Table 7, bracketed numbers), a similar trend occurred but a more stepwise progression of similarity was seen:

| | |
|---|---|
| C. occidentalis lab vs. McCall | .95 |
| C. fumiferana vs. C. occidentalis | .70-.72 |
| C. viridis vs. C. occidentalis | .50 |
| C. viridis vs. C. fumiferana | .34 |

2) Intraspecific Variation

a) Sibling group responses. In the 1977 survey of C. occidentalis and viridis sibling group responses to acephate (Tables 8-10), a series of parallel but not equal lines were demonstrated for both species. When these experiments were repeated in 1978 to compare the genetic composition of controls and survivors of insecticide treatments, stringent statistical standards were used to select the sibling groups used for the genetic/toxicological comparisons. Among 15 sibling groups selected at random from 100 available for each species, then treated with acephate, 10 C. occidentalis and 6 C. viridis groups had sufficiently precise response lines to be used for a genetic comparison.

The responses of the 10 C. occidentalis sibling groups (Table 11) were parallel (-2 log L-16.0;d.f.=9;p=0.067) but not equal (-2 log L =43.47;d.f.=18;p=0.001). The responses of the 6 C. viridis sibling groups (Table 12) were both parallel (-2 log L=3.19;d.f.=5;p0.671) and equal (-2 log L=18.18;d.f.=10;p=0.052).

Fifteen sibling groups from both the C. occidentalis and viridis laboratory colonies were also tested with carbaryl (Tables 13, 14). The responses of the C. occidentalis sibling groups were neither parallel nor equal; those of C. viridis were parallel but not equal (Table 15). No genetic analysis of survivors of these treatments was attempted because the dose-response lines could not be fixed with at least 90% certainty.

Gene frequencies of acephate-treated survivors in each sibling groups were compared to the pattern of toxicological response among the groups. Data from EST2 are shown in Tables 16 and 17. The genetic makeup of controls and survivors in the acephate-treated sibling groups was also compared. In 7 of the 10 C. occidentalis sibling groups, survivors had a higher frequency of allozyme #3 than did controls. When differences in frequency of genotypes 3-3 and 3-4 were calculated for treatment survivors vs. untreated controls, clear trends were noted (Table 16, B). With only one exception, susceptible groups were characterized by a $>30\%$ increase in genotype 3-3 among survivors, while the frequency of genotype 3-4 decreased $\gtrsim 27\%$. In two other groups (48, 51), no alteration in gene frequency was possible with treatment because the genotype of the sibling group was fixed for allozyme #3 in the entire group. In only one C. occidentalis sibling group (33) did the frequency change in favor of allozyme #4. Among selected acephate-treated C. viridis sibling groups, no trend of this type appeared. This observation was consistent with the absence of significant differences among the C. viridis sibling groups.

b) Comparison of laboratory and field populations of C. occidentalis.

Comparison of response of insects from the non-diapausing

laboratory colony and the McCall, Idaho, population (Table 1, A) to acephate showed that response lines were parallel (-2 log L=3.08;d.f. =1;p=0.079) but not equal (-2 log L=74.2;d.f.=2;p<0.001).

The genetic relationship between the two populations (Table 7) was consistent with intraspecific variation (e.g., Avise 1974).

c) Toxicological and genetic differences over C. occidentalis larval development. Toxicological responses of instars 2-6 to spray applications of acephate were equal (-2 log L=9.34;d.f.=8;p=0.312) and parallel (-2 log L=5.13;d.f.=4;p=0.274) (Table 18). Responses to carbaryl, however, were neither parallel (-2 log L=12.21;d.f.=4; p=0.016) nor equal (-2 log L=20.61;d.f.=8;p=0.008).

When esterase gene frequencies were compared among untreated instars 2-6 (Table 19), no differences were found at EST1. At EST2, the frequency of the common allozyme (#3) was lower in instars 4-5 than in other stages. At EST3, 3rd instars had a lower frequency of the common allozyme and a higher frequency of allozyme #3 than other stages. As genetic analyses of these instar tests are not yet complete, such differences can only be taken as indicative of general trends at this time.

Comparison of esterase allozyme frequencies among treated and untreated larvae are shown in Table 20. Larval stadia within treated and untreated groups are combined here for the purpose of an overall comparison of treated vs. control insect groups. No differences among these groups were seen at EST1. However, at EST2 a marked difference appeared between control (untreated) and survivor (treated) groups. In untreated larvae there was a higher proportion of the silent

allele and a much lower proportion of allozyme #3. At EST3, the carbaryl-treated group had a significantly higher proportion of the first allozyme and a lower percent of the common allozyme than either the acephate-treated or the untreated larvae.

## 5. CONCLUSIONS AND RECOMMENDATIONS

Significant differences in response to acephate occur both between and within species of spruce budworm. These differences must be considered in the course of developing chemical control programs in the various regions over which Choristoneura species are distributed.

A genetic basis for differential susceptibility is demonstrated by experiments with C. occidentalis and C. viridis sibling groups. Among these groups, significant differences to both acephate and carbaryl were noted in preliminary experiments. Because of the stringent statistical criteria used to select specific sibling groups for genetic and toxicological comparisons in the major portion of this investigation, we examined only acephate-treated groups in detail. A progression of toxicological responses was observed among the C. occidentalis sibling groups. Within this progression, significant differences in response were noted. These responses appeared to be associated with changes in the frequency of allozyme #3 at EST2. Extremely tolerant groups were characterized by 100% fixation for allozyme #3. Application of acephate appeared to favor survival of insects of genotype 3-3 at EST2, while those of genotype 3-4 or 4-4 were selected against. The exclusive presence of genotype 3-3 may be a useful marker for acephate tolerance. The feasibility of using this genotype as a marker should be considered in addi-

tional experiments with more sibling groups and other toxicants of different chemical types. In addition, the possible use of this allozyme to characterize relative population susceptibility should be explored. The potential use of such population markers has been discussed in another recent investigation (Stock and Robertson 1979). Finally, the apparent shift toward increased frequencies of genotype 3-3 at EST2 as the result of acephate treatment merits further investigation. If allozyme #3 at EST2 does prove to confer tolerance in followup tests, and application of acephate increases the frequency of insects of genotype 3-3, a mechanism for acquisition of increased population tolerance can be monitored in field studies.

The significant differences noted among larval stages of C. occidentalis sprayed with carbaryl in the form of Sevin-4-oil may also be related to EST2 allozyme frequencies. Until these genetic analyses are complete, however, definitive conclusions cannot be drawn.

## 6. WORK REMAINING ON STUDY

Genetic analyses of treated and untreated instars are not complete. These tests should be finished by December 1979. At that time, a full documentation of variation (toxicological and genetic) among C. occidentalis instars will be prepared for publication and submitted to CANUSA for review.

## 7. COOPERATION AND COORDINATION

We thank Dale Grisdale for providing diapausing second instar C. fumiferana for these studies, Lucille Boelter for assisting in the insecticide treatments, and Paul Castrovillo for help with the genetic analyses.

We also thank Northwest Airlines for not sending our insects to Minneapolis after mislabelling them to go there instead of to Spokane.

This work was another successful cooperative venture between the Pacific Southwest Forest & Rangœ Experiment Station and the University of Idaho, Department of Forest Resources. Each of the principal investigators attributes responsibility for errors in this work solely to the other.

8a. PROBLEMS ENCOUNTERED

The only really unique problem we encountered, other than the usual difficulties involved in doing this sort of research, was United Airlines going on strike the day that our insects were scheduled to be shipped from San Francisco to Spokane.

8b. SUGGESTIONS FOR FOLLOW-UP RESEARCH

This work has clearly shown that substantial differences in population response to acephate and carbaryl occur in C. occidentalis. It has also demonstrated the potential for applied genetic analysis in defining estimators of population response to these chemicals. This initial work should be expanded to include more populations and other chemicals currently being considered for control of the western spruce budworm. Populations may be selected from areas already scheduled for surveys of genetic variation and which are demonstrably different from each other at key esterase loci.

9. MANUSCRIPTS OR REPORTS PREPARED OR PLANNED

A manuscript covering the genetic and toxicological portions of this

research (species, populations, and sibling groups) is in the final stages of preparation and will be sent to CANUSA for review when it is completed. The instar portion of this work will be written up for publication as soon as it is completed (see #6 above).

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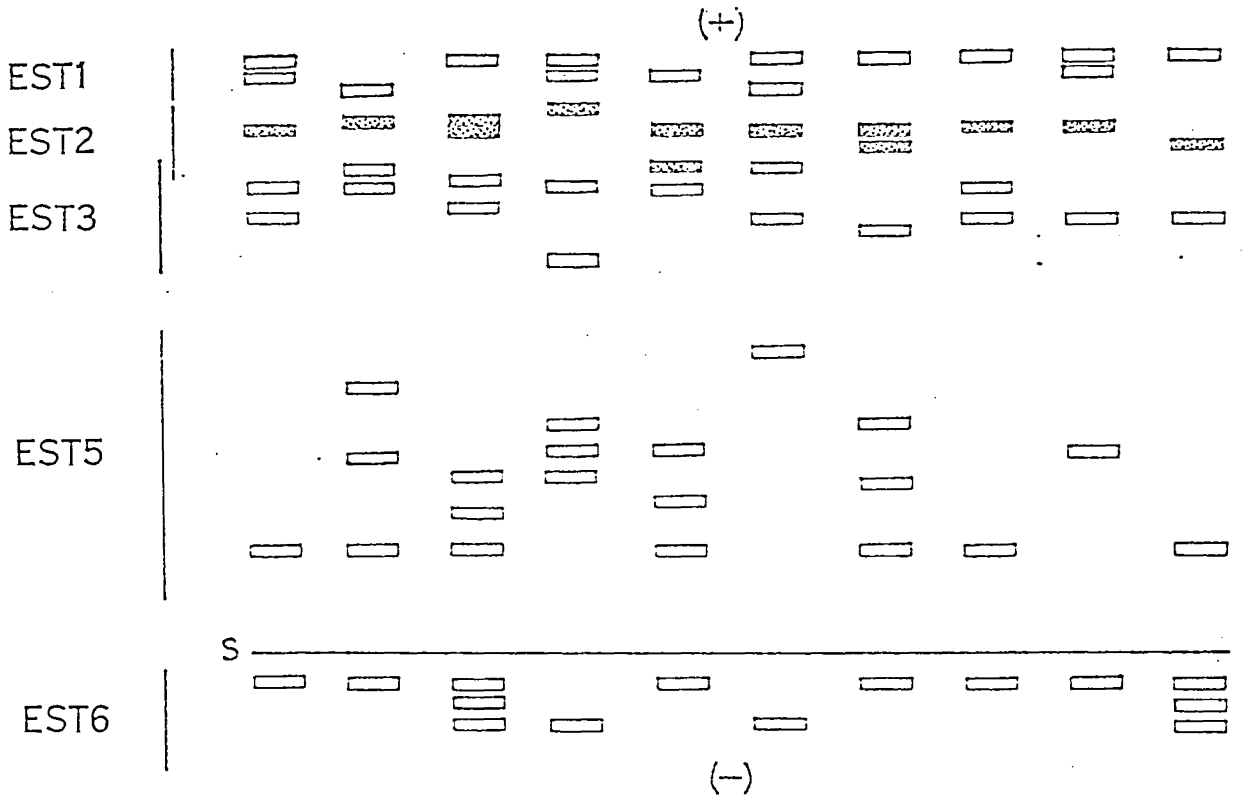


Figure 1. Diagram of the esterase enzyme system in the spruce budworm. Each vertical column of bands represent allozymes at 5 gene loci from a single insect (10 insects total).

Table 1. Comparative toxicity of acephate to Choristoneura species and populations.

| Species | N | NC | C ± S.E. | BETA ± S.E. | $LD_{50}$* | 95% CL* | $LD_{90}$* | 95% CL* | LOG L |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A. 1977 | | | | | |
| C. fumiferana | 299 | 34 | 0.125 ± 0.059 | 1.52 ± 0.31 | 32.6 | 14.5-54.0 | 228.1 | 113.4-1796 | -200.7 |
| C. occidentalis | | | | | | | | | |
| ND Colony | 1065 | 217 | 0.146 ± 0.022 | 2.02 ± 0.21 | 55.7 | 41.4-74.9 | 240.9 | 152.2-602.3 | -742.4 |
| McCall | 393 | 128 | 0.279 ± 0.039 | 2.71 ± 0.39 | 21.6 | 12.9-29.4 | 64.4 | 46.4-119.8 | -263.7 |
| C. viridis | 531 | 135 | 0.160 ± 0.034 | 1.64 ± 0.28 | 45.3 | 19.5-85.7 | 272.9 | 122.1-13900 | -397.1 |
| | | | | B. 1978 | | | | | |
| C. occidentalis | 953 | 201 | 0.110 ± 0.022 | 2.49 ± 0.24 | 38.7 | 32.6-44.8 | 126.5 | 101.5-174.9 | -607.0 |
| C. viridis | 915 | 173 | 0.254 ± 0.033 | 1.93 ± 0.27 | 42.2 | 28.7-56.1 | 193.9 | 127.0-453.3 | -671.4 |

* Dosage expressed as ug/g body weight.

Table 2. Comparative toxicity of carbaryl to Choristoneura occidentalis and C. viridis (1978).

| Species | N | NC | C ± S.E. | BETA ± S.E. | $LD_{50}$* | 95% CL* | $LD_{90}$* | 95% CL* | LOG L |
|---|---|---|---|---|---|---|---|---|---|
| C. occidentalis | 881 | 194 | 0.056 ± 0.016 | 0.56 ± 0.13 | 17.6 | 4.2-31.0 | 3305.1 | 47.6-4658 | -634.1 |
| C. viridis | 640 | 138 | 0.290 ± 0.039 | 0.89 ± 0.23 | 72.6 | 35.7-221.8 | 1969.5 | 44.9-6003 | -513.1 |

* Dosage expressed as ug/g body weight.

Table 3. Methods used in electrophoretic analysis of spruce budworm isozymes. (See Table 4 for enz. abbrev.)

| Enzyme | Buffer System | Migration Distance (cm) | Stain Recipe: Cofactor (5mg) | Other Components | Mixing Solution |
|---|---|---|---|---|---|
| AAT | RW[1] | 6 | - | 50mg Fast Garnet (GBC) salt | AAT buffer[2] |
| EST | RW | 6 | - | 1.5ml a-naphthylacetate[3]<br>1.5ml b-naphthylacetate[4]<br>5ml propanol<br>1 ml Fast Garnet solution[5] | Esterase buffer[6] |
| LAP | RW | 6 | - | 10mg L-leucyl beta naphthylamide<br>20 mg Fast Garnet (GBC) salt | 0.1 M monobasic phosphate buffer |
| PGM | RW | 4 | NADP | 50-100 mg glucose-1-phosphate<br>100 units glucose-1-6-diphosphate<br>100 units G6PDH<br>2 mg PMS<br>15 mg NBT | RW gel buffer |
| AcP | AC[7] | 6 | - | 1.4 g monobasic phosphate<br>2 mg a-naphthyl-acid phosphate<br>2 mg Fast Garnet GBC salt | Dist. water |
| LDH | AC | 6 | NAD | 20 ml 0.5M DL-Na-lactate, NBT, PMS | RW gel buffer |
| ME | AC | 4 | NADP | 20 ml 0.5M DL-Na-malate, NBT, PMS | RW gel buffer |
| TFN | RW | 4 | - | 50 mg Naphthol Blue Black | Gel fixative[8] |

TO (shows up on gels stained overlong with NBT; e.g., PGM, LDH, ME).

[1]Described by Ridgway et al. (1970). [2]600 ml RW stock + 900 ml dist. water + 2g a-ketoglutaric acid + 5g aspartic acid. [3] 0.2 g a-naphthylacetate + 10 ml dist. water + 10 ml acetone. [4] 0.2 g b-naphthylacetate + 10 ml dist. water + 10 ml acetone. [5] 0.8 g Fast Garnet + 20ml dist. water. [6]50 ml 'A' buffer (27.6 g monobasic NaPO4 + 1000 ml dist. water) + 10 ml 'B' buffer (28.2 g anhydrous dibasic Na2HPO4 + 1000 ml dist. water) + 40 ml dist. water. [7]Described by Shaw and Prasad (1970). [8] 5.5:5:1 mixture of 95% ethanol, distilled water and glacial acetic acid.

Table 4. General characteristics of enzymes assayed in C. occidentalis, viridis, and fumiferana.

| Name | Abbrev. | Locus | Anodal or Cathodal | Molecular Form (dimer or monomer) | Polymorphic or Monomorphic | Number of Allozymes |
|---|---|---|---|---|---|---|
| Aspartate aminotransferase | AAT | 1 | A | D | P | 3 |
| | | 2 | C | ? | M | 1 |
| Esterase | EST | 1 | A | M | P PP | 4 |
| | | 2 | A | M | P | 5 (one silent) |
| | | 3 | A | M | P | 5 |
| | | 4 | A | M | P | 2* |
| | | 5 | A | D | P | 7 |
| | | 6 | C | D | P | 2 |
| Leucylaminopeptidase | LAP | 1 | A | M | P | 2* |
| | | 2 | A | M | P | 2 |
| | | 3 | A | M | P | 2 |
| Phosphoglucomutase | PGM | 1 | A | M | P | 4* |
| Acid phosphatase | AcP | 1 | A | ? | M | 1 |
| Lactate dehydrogenase | LDH | 1 | A | ? | M | 1 |
| Malic Enzyme | ME | 1 | A | ? | M | 1 |
| Transferrin | TFN | 1 | A | ? | M | 1 |
| Tetrazolium oxidase | TO | 1 | A | ? | M | 1 |

* Not consistently resolved.

Table 5. Characteristics of esterase isozymes in the spruce budworm.

| Locus Designation | Direction of Movement | Molecular Structure | Allozyme ("Allele") Designation* | $r_f$ Value of Common Allele | Comments |
|---|---|---|---|---|---|
| EST1 | anodal | monomer | 104** | | Stains blue. |
| | | | 100 | .91 | |
| | | | 98 | | |
| | | | 96 | | |
| EST2 | anodal | monomer | 109** | | Sensitive to b-naphthylacetate substrate (stains red). |
| | | | 104 | | |
| | | | 100 | .57 | |
| | | | 95 | | |
| | | | 89** | | |
| EST3 | anodal | monomer | 112** | | Stains blue. |
| | | | 107 | | |
| | | | 100 | .43 | |
| | | | 95 | | |
| | | | 91*** | | |
| EST5 | anodal | dimer | 567** | | Stains blue. |
| | | | 467 | | |
| | | | 400 | | |
| | | | 350 | | |
| | | | 300 | | |
| | | | 100 | .06 | |
| | | | 33** | | |
| EST6 | cathodal | dimer | 333 | | Stains blue. |
| | | | 200 | | |
| | | | 100 | .06 | |

*Common allele is 100; other alleles designated by mobility relative to common allele.
**Rare.
***Rare in C. occidentalis and fumiferana; common in C. viridis laboratory colony.

Table 6. Isozyme frequencies in untreated laboratory and field populations

| Locus | Allozyme | CO Lab | CO McCall | CV Lab | CF Lab |
|---|---|---|---|---|---|
| EST2 | p(1) | 0 | 0 | 0 | 0 |
| | p(2) | .06 | .02 | .48 | .02 |
| | p(3) | .80 | .71 | .43 | .90 |
| | p(4) | .02 | .08 | 0 | .03 |
| | p(5) | 0 | 0 | 0 | 0 |
| | p(S) | .12 | .19 | .09 | .05 |
| | N | 256 | 52 | 119 | 82 |
| EST3 | p(1) | .\0 | 0 | 0 | 0 |
| | p(2) | 0 | 0 | 0 | 0 |
| | p(3) | .98 | .95 | 0 | .85 |
| | p(4) | .02 | .05 | 0 | .11 |
| | p(5) | 0 | 0 | 1.0 | .04 |
| | N | 255 | 54 | 120 | 50 |
| EST5 | p(1) | 0 | 0 | 0 | 0 |
| | p(2) | .01 | 0 | 0 | 0 |
| | p(3) | 0 | .01 | 0 | .62 |
| | p(4) | .01 | .02 | 0 | .08 |
| | p(5) | .12 | .08 | .23 | .10 |
| | p(6) | .86 | .89 | .77 | .20 |
| | N | 188 | 83 | 97 | 74 |
| AAT1 | p(1) | 0 | 0 | 0 | .70 |
| | p(2) | 0 | 0 | 0 | .28 |
| | p(3) | 1.0 | 1.0 | 1.0 | .02 |
| | N | 231 | 49 | 114 | 110 |
| LAP1 | p(1) | .49 | -- | .85 | -- |
| | p(2) | .51 | -- | .15 | -- |
| | N | 197 | -- | 57 | -- |
| LAP2 | p(1) | .72 | -- | .59 | .72 |
| | p(2) | .28 | -- | .41 | .28 |
| | N | 221 | -- | 32 | 47 |
| LAP3 | p(1) | .23 | 0 | .07 | .05 |
| | p(2) | .77 | 1.0 | .93 | .95 |
| | N | 216 | 76 | 165 | 56 |
| PGM | p(1) | .13 | -- | .24 | -- |
| | p(2) | .34 | -- | .09 | -- |
| | p(3) | .33 | -- | .53 | -- |
| | p(4) | .20 | -- | .14 | -- |
| | N | 293 | -- | 89 | -- |
| AAT2 | N | 226 | 48 | 112 | 110 |
| EST6 | N | 210 | 51 | 105 | 76 |
| LDH | N | 183 | 44 | 90 | 45 |
| ME | N | 195 | 50 | 88 | 48 |
| TFN | N | 230 | 54 | 118 | 103 |
| AcP | N | 72 | 27 | 30 | 32 |
| TO | N | 114 | 46 | 62 | 58 |

Table 7. Rogers' similarity coefficients calculated between populations of spruce budworm over all gene loci and over esterase loci only (in parentheses).

| | *C. occidentalis* | | *C. viridis* | *C. fumiferana* |
|---|---|---|---|---|
| | Lab Colony | McCall | Lab Colony | Lab colony |
| *C. occidentalis* | | | | |
| Lab Colony | --- | | | |
| McCall | .97 (.95) | --- | | |
| *C. viridis* | | | | |
| Lab Colony | .84 (.50) | .87 (.50) | --- | |
| *C. fumiferana* | | | | |
| Lab Colony | .85 (.72) | .85 (.70) | .77 (.34) | --- |

Table 8. Sibling Group Responses of C. occidentalis to Acephate (1977).

| Egg Mass # | N | NC | C ± S.E. | BETA ± S.E. | $LD_{50}$ [a] | $LD_{90}$ [a] | LOG L |
|---|---|---|---|---|---|---|---|
| 29 | 70 | 20 | 0.193 ± 0.075 | 1.77 ± 1.25 | (134.1) | (708.1) | -52.4 |
| 13 | 82 | 15 | 0.331 ± 0.079 | 3.48 ± 1.43 | (59.4) | (138.9) | -60.3 |
| 17 | 89 | 18 | 0.144 ± 0.073 | 2.05 ± 0.66 | (52.1) | (219.8) | -62.0 |
| 12 | 79 | 16 | 0.130 ± 0.087 | 1.07 ± 0.58 | (65.7) | (1034.5) | -58.3 |
| 10 | 149 | 25 | 0.075 ± 0.047 | 2.34 ± 0.51 | (38.9) | (137.2) | -77.2 |
| 14 | 108 | 21 | 0.182 ± 0.063 | 2.79 ± 0.68 | 44.4 | 127.7 | -72.4 |
| 4 | 66 | 12 | 0.177 ± 0.080 | 2.65 ± 1.16 | (66.7) | (202.7) | -44.9 |
| 33 | 68 | 12 | 0 ± 0 | 1.98 ± 0.53 | 31.3 | 138.9 | -39.1 |
| 39 | 50 | 10 | 0.340 ± 0.067 | 15.10 ± 730.42 | (104.2) | (126.7) | -38.8 |
| 24 | 57 | 12 | 0.076 ± 0.072 | 0.77 ± 0.63 | (316.0) | (14337.8) | -36.5 |
| 25 | 109 | 20 | 0 ± 0 | 1.90 ± 0.41 | 36.1 | 171.2 | -63.4 |
| 1 | 58 | 14 | 0.151 ± 0.100 | 1.37 ± 1.15 | (117.6) | (1011.3) | -42.3 |
| 28 | 101 | 23 | 0.097 ± 0.049 | 3.21 ± 0.68 | 40.1 | 100.6 | -59.0 |

[a] Dosage expressed in ug/g body weight. Parentheses indicate that no 90-95% confidence limits could be defined

Table 9. Response of C. viridis sib. to acephate (1977).

| Egg Mass # | N | NC | C ± S.E. | BETA ± S.E. | $LD_{50}$ a) | $LD_{90}$ a) | LOG L |
|---|---|---|---|---|---|---|---|
| 23 | 48 | 11 | 0.080 + 0.074 | 1.94 ± 0.72 | (42.7) | (195.0) | -31.9 |
| 31 | 36 | 9 | 0.123 ± 0.120 | 1.18 ± 0.88 | (41.0) | (497.8) | -26.8 |
| 15 | 48 | 9 | 0 ± 0 | 1.44 ± 0.58 | (32.6) | (251.9) | -29.9 |
| 16 | 70 | 15 | 0.132 ± 0.087 | 1.60 ± 0.59 | (23.7) | (150.2) | -48.1 |
| 26 | 38 | 9 | 0.327 ± 0.095 | 4.58 ± 2.98 | (65.3) | (124.3) | -28.8 |
| 3 | 100 | 24 | 0.083 ± 0.056 | 2.02 ± 0.50 | 25.9 | 111.6 | -64.2 |
| 44 | 40 | 10 | 0.310 ± 0.071 | 16.01 | (101.6) | (122.1) | -31.3 |
| 27 | 45 | 9 | 0.173 ± 0.080 | 4.71 ± 1.97 | (57.6) | (107.8) | -27.5 |

a) Dosage expressed in ug/g body weight. Parentheses indicate that no 90-95% confidence limits could be computed.

Table 10. Test of Acephate Sibling Groups - C. O. & C. V. 1977.

| | Parallelism | | | Equality | | |
|---|---|---|---|---|---|---|
| | d.f. | -2 log λ | p | d.f. | -2 log λ | p |
| CO | 12 | 14.89 | 0.248* | 24 | 62.05 | 0 |
| CV | 7 | 7.93 | 0.339* | 14 | 37.07 | .001 |

Table 11. Toxicity of acephate to sibling groups of C. occidentalis (1978)

| Group No. | N | NC | C±S.E. | BETA±S.E. | $LD_{50}$ [a] | CL [b] | $LD_{90}$ [a] | CL [b] | LOG L |
|---|---|---|---|---|---|---|---|---|---|
| 47 | 82 | 17 | 0±0 | 1.99±0.49 | 21.1 | 9.7-32.3 | 93.0 | 53.8-516.1 | -44.2 |
| 42 | 65 | 14 | 0±0 | 1.84±0.54 | 26.5 | (14.1-44.7) | 131.7 | (66.3-1811.8) | -38.3 |
| 33 | 65 | 12 | 0±0 | 2.58±0.61 | 27.8 | 17.6-41.6 | 87.1 | 53.8-340.7 | -33.4 |
| 36 | 66 | 13 | 0±0 | 1.28±0.48 | 29.1 | (15.3-51.1) | 292.9 | (115.0-1138) | -42.0 |
| 4 | 58 | 13 | 0±0 | 2.27±0.62 | 31.5 | 19.5-50.1 | 115.5 | 65.5-734.9 | -32.3 |
| 30 | 112 | 20 | 0.046±0.044 | 2.24±0.48 | 33.4 | 17.9-59.5 | 125.1 | 67.1-1223.4 | -67.1 |
| 24 | 103 | 21 | 0.151±0.063 | 3.62±1.13 | 38.9 | 22.1-51.6 | 87.9 | 63.7-245.8 | -61.0 |
| 17 | 43 | 12 | 0±0 | 3.38±0.97 | 39.1 | 28.1-61.1 | 93.5 | 60.2-361.8 | -19.1 |
| 48 | 70 | 16 | 0.096±0.052 | 4.55±1.37 | 46.2 | 30.3-61.4 | 88.4 | 65.7-206.8 | -35.4 |
| 51 | 94 | 22 | 0.042±0.035 | 3.15±0.79 | 53.3 | 38.8-75.8 | 135.9 | 90.4-384.2 | -45.7 |

a> Dosage expressed in µg/g. body weight.

b> Limits in parentheses are 90% confidence limits. All others are 95% confidence limits.

Table 12. Toxicity of acephate to C. viridis sibling groups (1978)

| Group No. | N | NC | C±S.E. | BETA±S.E. | $LD_{50}$ a> | CL b> | $LD_{90}$ a> | CL b> | LOG L |
|---|---|---|---|---|---|---|---|---|---|
| 9 | 77 | 12 | 0±0 | 2.62±0.60 | 19.0 | 10.2-27.9 | 58.6 | 37.1-248.1 | -38.1 |
| 45 | 64 | 14 | 0±0 | 1.77±0.60 | 19.2 | 8.2-29.9 | 101.9 | 53.0-1731.3 | -38.3 |
| 20 | 66 | 12 | 0±0 | 2.89±0.62 | 24.3 | 13.8-39.7 | 67.5 | 40.9-372.4 | -31.6 |
| 29 | 62 | 10 | 0±0 | 2.39±0.63 | 27.4 | 15.3-46.4 | 93.8 | 52.5-997.8 | -34.2 |
| 4 | 79 | 16 | 0.136±0.088 | 2.43±0.77 | 29.6 | (8.65-48.1) | 99.7 | (58.9-849.5) | -50.1 |
| 7 | 68 | 9 | 0±0 | 1.75±0.51 | 48.6 | (27.6-103.9) | 241.9 | 103.8-7081.6 | -39.5 |

a> Dosage in µg/g body weight.

b> Limits in parentheses are 90% confidence limits. All others are 95% confidence limits.

Table 13. Toxicity of Carbaryl to C. occidentalis Sibling Groups (1978).

| | N | NC | C ± S.E. | BETA ± S.E. | $LD_{50}$ | $LD_{90}$ | LOG L |
|---|---|---|---|---|---|---|---|
| 21 | 62 | 9 | 0.091 ± 0.085 | -0.91 ± 0.57 | (9.3) | (0.4) | -42.7 |
| 31 | 66 | 16 | 0.128 ± 0.085 | 1.41 ± 0.70 | (62.8) | (506.9) | -47.7 |
| 14 | 65 | 14 | 0 | 0.69 ± 0.50 | (19.1) | (1348.9) | -43.7 |
| 27 | 75 | 18 | 0.055 ± 0.053 | 0.55 ± 0.45 | (7.7) | (1639.9) | -51.5 |
| 38 | 54 | 9 | 0 | 0.98 ± 0.56 | (38.9) | (795.7) | -35.7 |
| 3 | 28 | 6 | 0.193 ± 0.201 | 1.78 ± 2.11 | (63.7) | (334.6) | -20.7 |
| 49 | 47 | 11 | 0 | 0.22 ± 0.54 | (6.7) | 5165032.7 | -32.2 |
| 32 | 27 | 5 | 0 | -.02 ± 0.73 | (0.8028) | | -18.5 |
| 28 | 83 | 19 | 0.052 ± 0.051 | -.63 ± 0.45 | (104.4) | 1.00 | -56.6 |
| 29 | 46 | 11 | 0.104 ± 0.099 | 2.11 ± 1.07 | (50.8) | 206.1 | -30.2 |
| 25 | 21 | 5 | 0.400 ± 0.219 | 0.04 ± 1.04 | (0.9) | | -15.9 |
| 46 | 84 | 18 | 0 | 1.69 ± 0.61 | (5.8) | (32.9) | -33.0 |
| 53 | 65 | 19 | 0.103 ± 0.068 | 1.43 ± 0.65 | (46.4) | (363.8) | -47.9 |
| 70 | 67 | 16 | 0 | 0.75 ± 0.47 | (10.4) | (527.4) | -43.4 |
| 55 | 91 | 18 | 0 | 0.84 ± 0.46 | (5.8) | (192.1) | -51.8 |

Table 14. Responses of C. viridis Sibling Groups to Carbaryl (1978).

| | N | NC | C ± S.E. | BETA ± S.E. | $LD_{50}$ | $LD_{90}$ | LOG L |
|---|---|---|---|---|---|---|---|
| 10 | 50 | 11 | 0.228 ± 0.072 | 4.32 ± 2.57 | (83.3) | (164.9) | -34.7 |
| 28 | 38 | 9 | 0.240 ± 0.173 | 0.79 ± 1.29 | (102.3) | (4320.8) | -30.8 |
| 60 | 35 | 7 | 0.142 ± 0.132 | 0.53 ± 0.72 | (22.2) | (5554.8) | -26.1 |
| 24 | 78 | 17 | 0.340 ± 0.108 | 0.98 ± 0.74 | (114.4) | (2317.2) | -63.8 |
| 15 | 57 | 9 | 0.109 ± 0.102 | 0.60 ± 0.55 | (27.9) | (3849.2) | -41.3 |
| 12 | 45 | 9 | 0.215 ± 0.134 | 0.61 ± 0.65 | (36.3) | (4431.1) | -34.6 |
| 47 | 39 | 9 | 0.475 ± 0.079 | 14.21 | (109.6) | (134.9) | -33.0 |
| 13 | 73 | 18 | 0.422 ± 0.067 | 7.73 ± 7.44 | (76.4) | (111.9) | -55.6 |
| 72 | 66 | 14 | 0.515 ± 0.098 | 3.41 ± 1.97 | (43.7) | (103.7) | -46.0 |
| 66 | 17 | 3 | 0 | -.46 ± 0.87 | (8.6) | 0 | -11.4 |
| 73 | 62 | 13 | 0.212 ± 0.103 | 1.23 ± 0.75 | (95.0) | (1047.5) | -47.8 |
| 61 | 29 | 8 | 0 | 0.30 ± 0.67 | (153.0) | (3369291.1) | -19.6 |

35, 40, 70 removed from sample had ≥ 50% check mortality.

Table 15. Tests of Equality and Parallelism of Sibling Groups to Carbaryl (1978).

| Group | Parallelism | | | Equality | | |
|---|---|---|---|---|---|---|
| | d.f. | -2 log λ | P | d.f. | -2 log λ | P |
| C.O. sibs- | 14 | 29.97 | 0.008 | 28 | 122.44 | <0.001 |
| C.V. sibs | 11 | 18.85 | 0.064* | 22 | 37.37 | 0.022 |

* indicates hypothesis accepted

Table 16. Gene frequencies at EST2 and insecticide response for acephate-treated C. occidentalis sibling groups.

| Sibling Group | | 47 | 42 | 33 | 36 | 4 | 30 | 24 | 17 | 48 | 51 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $LD_{50}$ | | 21.1 | 26.5 | 27.8 | 29.1 | 31.5 | 33.4 | 38.9 | 39.1 | 46.2 | 53.3 |
| | | | | | A. Frequencies in treated and untreated groups. | | | | | | |
| Frequency in treatment survivors: | p(3-3) | .54 | .80 | .14 | .84 | .86 | .92 | .32 | .33 | 1.00 | 1.00 |
| | p(3-4) | .23 | .11 | .21 | .14 | .12 | .07 | .40 | .33 | 0 | 0 |
| | p(4-4) | .23 | .09 | .65 | .02 | .02 | .01 | .28 | .33 | 0 | 0 |
| Frequency in untreated controls: | p(3-3) | .24 | .24 | .27 | .49 | .53 | .50 | .25 | .20 | 1.00 | 1.00 |
| | p(3-4) | .55 | .52 | .48 | .50 | .45 | .49 | .46 | .45 | 0 | 0 |
| | p(4-4) | .21 | .24 | .25 | .01 | .02 | .01 | .29 | .35 | 0 | 0 |
| | | | | | B. Differences in frequencies (treated minus controls). | | | | | | |
| Genotype | 3-3 | +0.30 | +0.56 | -0.13 | +0.35 | +0.33 | +0.42 | +0.07 | +0.13 | 0 | 0 |
| | 3-4 | -0.32 | -0.41 | -0.27 | -0.36 | -0.33 | -0.42 | -0.06 | -0.12 | 0 | 0 |
| | 4-4 | +0.02 | <0.05 | +0.40 | +0.01 | 0 | 0 | -0.01 | -0.03 | 0 | 0 |

Table 17. Gene frequencies/insecticide response for C. viridis sibling group survivors of acephate treatments.

| | Sibling Group | 9 | 45 | 29 | 4 |
|---|---|---|---|---|---|
| | $LD_{50}$ | 19.0 | 19.2 | 27.4 | 29.6 |
| EST2 genotype: | 3-3 | 39 | 14 | 11 | 40 |
| | 3-4 | 10 | 20 | 14 | 16 |
| | 4-4 | 0 | 20 | 20 | 6 |
| | N | 49 | 54 | 45 | 62 |
| Frequency in treatment survivors: | p(3) | .90 | .44 | .40 | .77 |
| | p(4) | .10 | .56 | .60 | .23 |
| Frequency in untreated controls: | p(3) | .72 | .54 | .44 | .45 |
| | p(4) | .28 | .46 | .56 | .55 |

Table 18. Comparative response of C. occidentalis instars to acephate (Orthene 755) and carbaryl (Sevin-4-oil) sprays.

| Instar | N | NC | C±S.E. | BETA±S.E. | $LD_{50}$ [a)] | 95%CL | $LD_{90}$ | 95%CL | LOG L |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A. Acephate | | | | | |
| 2 | 400 | 40 | 0.144±0.24 | 4.34±0.63 | 51.7 | 42.8-60.4 | 102.0 | 84.5-137.9 | -193.6 |
| 3 | 400 | 40 | 0.121±0.028 | 3.54±0.63 | 52.6 | 41.6-62.0 | 120.9 | 98.0-175.9 | -195.3 |
| 4 | 400 | 50 | 0.058±0.018 | 3.40±0.42 | 50.1 | 42.7-57.9 | 119.1 | 97.6-159.9 | -167.3 |
| 5 | 400 | 50 | 0 | 3.09±0.27 | 44.2 | 36.2-55.0 | 114.9 | 86.0-180.6 | -131.7 |
| 6 | 320 | 50 | 0 | 3.14±0.29 | 47.9 | 39.4-59.5 | 122.9 | 92.1-194.8 | -127.5 |
| | | | | B. Carbaryl | | | | | |
| 2 | 400 | 50 | 0.104±0.049 | 1.95±0.30 | 53.7 | 18.8-83.6 | 244.0 | 145.8-1178 | -241.7 |
| 3 | 400 | 50 | 0.101±0.042 | 2.02±0.30 | 64.8 | 48.6-80.4 | 280.4 | 207.0-4633 | -253.3 |
| 4 | 400 | 50 | 0.043±0.030 | 1.73±0.26 | 69.3 | 45.2-94.3 | 383.8 | 229.5-1286 | -256.8 |
| 5 | 400 | 50 | 0.081±0.039 | 1.33±0.26 | 70.4 | 47.6-94.6 | 651.2 | 357.3-2341 | -272.9 |
| 6 | 400 | 50 | 0 | 1.01±0.22 | 62.6 | 31.6-95.0 | 1159.3 | 399.6-76700 | -265.4 |

a) Dosage expressed as g/ha.

Table 19. Esterase allozyme frequencies in untreated western spruce budworm instars 2-6.

| Locus | Frequencies | Instar: 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| EST1 | p(1) | .52 | .65 | .57 | .68 | .55 |
| | p(2) | .48 | .35 | .43 | .32 | .43 |
| | p(3) | 0 | 0 | .01 | 0 | .01 |
| | p(4) | 0 | 0 | 0 | 0 | 0 |
| | N | 31 | 49 | 80 | 48 | 168 |
| EST2 | p(1) | 0 | 0 | 0 | 0 | 0 |
| | p(2) | .02 | .02 | .02 | .02 | 0 |
| | p(3) | .83 | .78 | .75 | .75 | .86 |
| | p(4) | .03 | .05 | .04 | .07 | .04 |
| | p(5) | 0 | 0 | 0 | 0 | 0 |
| | p(S) | .12 | .15 | .19 | .12 | .10 |
| | N | 202 | 123 | 290 | 255 | 504 |
| EST3 | p(1) | 0 | .09 | 0 | 0 | .02 |
| | p(2) | .02 | 0 | 0 | 0 | 0 |
| | p(3) | .98 | .91 | 1.00 | .99 | .98 |
| | p(4) | 0 | 0 | 0 | 0 | 0 |
| | p(5) | 0 | 0 | 0 | 0 | 0 |
| | N | 31 | 38 | 73 | 241 | 410 |

Table 20. Comparison of esterase allozyme frequencies among treated and untreated western spruce budworm larvae.

| Locus | Frequency | Acephate | Carbaryl | Untreated |
|---|---|---|---|---|
| EST1 | p(1) | 0 | 0 | . 0 |
| | p(2) | .56 | .61 | .58 |
| | p(3) | .44 | .39 | .41 |
| | p(4) | 0 | 0 | .01 |
| | N | 8 | 19 | 374 |
| EST2 | p(S) | 0 | 0 | .16 |
| | p(1) | 0 | 0 | 0 |
| | p(2) | 0 | .03 | .01 |
| | p(3) | .95 | .97 | .78 |
| | p(4) | .02 | 0 | .05 |
| | p(5) | .02 | 0 | 0 |
| | N | 44 | 35 | 1374 |
| EST3 | p(1) | 0 | .21 | .02 |
| | p(2) | 0 | 0 | 0 |
| | p(3) | 1.0 | .79 | .98 |
| | p(4) | 0 | 0 | 0 |
| | p(5) | 0 | 0 | 0 |
| | N | 22 | 21 | 803 |

CANADA/U.S. SPRUCE BUDWORMS PROGRAM - WEST

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# Genetic Features of *Choristoneura occidentalis*, *C. viridis*, and *C. fumiferana* Populations Exhibiting Differential Response to Insecticides


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1. SUMMARY

Comparisons of toxicological response to carbaryl (Sevin) and acephate (Orthene) and genetic makeup of treated and untreated groups were made among instars 2-6 of the western spruce budworm, Choristoneura occidentalis Freeman. No significant differences occurred, over larval development, in response to acephate but a shift toward greater tolerance through development was observed with carbaryl. In later instars (5th-6th), both carbaryl and acephate appear to select for insects with EST2 allozyme 3, though in younger instars (2nd-3rd) the reverse appears to be true. Genetic data also suggest that the frequency of the silent EST2 allele diminishes as a direct result of treatment with either toxicant. Selection against the silent allele appears uniform over all instars when acephate is used. For carbaryl, the greatest shift in favor of the active EST2 alleles occurs in 6th instars. The frequency of silent alleles in western spruce budworm populations appears to be associated with insecticide tolerance. Tests of the utility of using genetic markers to detect tolerance in field populations would be an important step toward improving effectiveness of decisions related to use of carbaryl and acephate for control of the western spruce budworm.

## 2. INTRODUCTION

The overall objective of this work was to examine in detail both inter- and intraspecific variation in toxicological response in selected Choristoneura species. We sought to define the underlying genetic basis for any observed differences in response and to identify genetic markers which might aid in predicting the response of spruce budworm species to insecticides before the insecticides are applied in a control program. A final report describing this work was submitted on 1 November 1979. One portion of the work was incomplete and not included in that final report. This was the portion of the study concerned with intraspecific variation in insecticide response in C. occidentalis, the western spruce budworm. A previous investigation of spray and feeding toxicities of acephate (Orthene) and carbaryl (Sevin) to instars 1-6 of the western spruce budworm revealed small differences in susceptibility to acephate but large differences in susceptibility to carbaryl (Robertson 1980). Our objective was therefore to:

2c) Compare toxicological response of different ~~larval~~ instars of C. occidentalis to acephate and carbaryl and to look for differences in esterase expression that might be associated with alterations in response during development.

The results of this portion of our investigation are described in this report.

## 3. METHODS

To determine the dosage range for each instar, each of 3 dosages (0.1, 1, 10 mg/ml), plus the solvent, were sprayed on 10 insects. Mortality was tallied after 7 days. These preliminary data were used to select 8 dosages which encompassed mortalities ranging from ca. 10-90%.

For the full-scale bioassay, each test was replicated 3 times with the 8 dosages using 20 insects per dosage in each replication. A control group of 20 treated with solvent only was tested with each replication. Therefore, each replication included 180 insects treated with insecticide, plus 10 control insects. A complete test with each instar included 480 insects and 60 controls (total 540). Insects of the appropriate instar were selected at random from the 77-80th generations of the non-diapausing laboratory colony. Instars were determined as described by Lyon et al. (1972).

The toxicity of Orthene 75S in water and Sevin-4-oil in diesel oil was tested using the design outlined above. Insects were sprayed in groups of 10 after they had been anesthetized with $CO_2$. After 60 seconds of exposure to the spray, each group of 10 was placed in a filter paper lined, sterile plastic petri dish with artificial diet.

For dosage determination, colorimetric analysis, described by Raynor (1956) was used. Oil red dye (1% w/v) was added to the Sevin-4-oil formulations, aniline blue (1% w/v) to the Orthene formulations. One deposit estimation per replication with each chemical tested on each instar was collected. Water was used in elution of the aniline blue deposits, toluene with the oil red deposits. Average deposit estimates will be used to determine dosage in oz/acre using the formula:

$$\frac{\text{weight of dye (ug) x conc. of insecticide (ug/ul)}}{\text{area of filter paper (63.6 cm}^2\text{) x conc. of dye (ug/ul)}} \div 0.7$$

The stainless steel spray chamber described by Robertson et al. (1978) was used for spray application. A uniform reservoir volume of 0.25 ml. equivalent to 1 gal/acre of applied spray, was sprayed.

In feeding tests, technical grade acephate and carbaryl were formulated in reagent grade acetone. Two hundred ul of each concentration were added to

10 ml of liquified artificial diet and the diet stirred thoroughly. When hardened, each diet mold was fed to 10 larvae (Robertson 1978).

Did you try just acetone too as a control?

Mortality was tallied 7 days post-treatment and survivors were subjected to electrophoretic separation of proteins followed by histochemical staining to reveal patterns of variation in the esterases. Methods of electrophoresis followed these described by Willhite (1979). Frozen insects were homogenized individually in small test tubes, centrifuged for 5 mins and the supernatent abosrbed onto small (3 x 13 mm) paper wicks which were stored frozen in Parafilm envelopes. Modifications of this method were used for instars 2-3.

Gels were made from a 13% hydrolyzed potato starch and buffer solution which was boiled, degassed under vacuum, poured into molds, and allowed to harden and cool for at least one hour. Wicks were inserted in a lengthwise slice in the gel approximately 3 cm from the cathodal end with minimal (i.e., 1 mm) spacing and punctuated at each end and between each 10 insect wicks, with a wick containing a dye marker (dilute food coloring). The dye marked the movement of proteins across the gel during electrophoresis. Appropriate safety devices (e.g., covers, warning signs) were used. During electrophoresis, a constant current of 75 mA and a maximum of 400V was maintained. Electrophoresis continued until the dye had migrated 6-9 cm from the slice.

Following electrophoresis, 1/16th inch (1.5 mm) thick horizontal slices of gel were stained. Esterases were resolved using a Ridgway (Ridgway et al. 1970) discontinuous buffer system during electrophoresis and a stain solution containing both alpha- and beta-naphthylacetate substrates (described by Bush and Huettel 1972).

Mortality data were subjected to probit analysis using POLO (Russell et al. 1977). The response of the instars to each toxicant applied in each

manner was compared with likelihood ratio tests (Savin et al. 1977).

The genetic makeup of each budworm group, expressed as genotype and allelic frequencies for each of the 5 polymorphic esterase loci from which data were obtained, was compared to expected values derived from the Hardy-Weinberg equation with the chi-square test. To test for significant differences in gene frequencies at each locus between groups, a contingency chi-square, based on the observed number of each allele in each group, was calculated.

## 4. RESULTS

Toxicological responses of instars 2-6 to spray applications of acephate were equal (-2 log L=9.34; d.f.=8; p=0.312) and parallel (-2 log L =5.13; d.f.=4; p=0.274) (Table 1). In other words, no significant differences in response occurred, over development, in response to acephate. Responses, to carbaryl, however, were neither parallel (-2 log L=12.21; d.f.=4; p=0.016) nor equal (-2 log L=20.61; d.f.=8; p=0.008). That is, significant differences did occur, over larval development, in response to carbaryl. At $LD_{50}$, the increasing order of tolerance was instar 2, 6, 3, 4, 5. At $LD_{90}$, however, the order of tolerance shifted slightly because of differences in slopes of the response lines. Increasing order of tolerance at $LD_{90}$ was 2, 3, 4, 5, 6.

Repeat? I'd try again.

Genetic data were obtained for esterase loci 1, 2, 3, 5, and 6. (Esterase locus 4 only shows up sporadically and consistent interpretation of this locus has not been possible.) In the western spruce budworm, esterase loci 5 and 6 do not show high levels of variation, especially in the laboratory population, while loci 1, 2, and 3 are highly polymorphic. Therefore, in this study, attention was focused on comparisons of instars and untreated vs. treated groups at the EST1, 2, and 3 loci.

Data obtained for EST1 and EST3 revealed no significant or consistent changes over larval development and when treated and untreated groups were compared. However, some interesting and relevant differences were seen when EST2 data were compared among groups. Because of the relative rarity of occurrence of allozymes 1, 2, and 5 at EST2 in these colony insects, data are only shown for frequencies of EST2 allozymes 3 and 4.

Hardy-Weinberg tests are used to detect deviations from proportions of genotypes expected when random mating occurs among all members of a group. Deviations from Hardy-Weinberg proportions (i.e., high chi-square values) occur when factors such as selection, inadequate sample size, the Wahlund effect (pooling of subsamples), or the presence of a silent allele disrupts the theoretical equilibrium. Hardy-Weinberg tests revealed that, in all untreated groups, there was a deficiency of heterozygotes (genotype 3-4) and an excess of homozygotes (genotypes 3-3 and 4-4) at the EST2 locus. For example, in the untreated carbaryl controls, instar 2, nine 3-4s were observed and almost 34 were expected. This trend was consistent through all instars in both carbaryl and acephate control groups and resulted in large and highly significant chi-square values (Table 2).

Such a trend (i.e., excess homozygotes, insufficient heterozygotes) is consistent with there being a high proportion of a "silent" allele present in the untreated laboratory populations. A silent allele at EST2 has been previously reported by Willhite (1979) and Robertson and Stock (1980). A silent allele produces an inactive form of the enzyme which does not show up as a band on the gel; thus, the presence of a silent allele can only be inferred.

The proportion of insects carrying the silent allele is much less in the treated groups than the untreated groups (Table 2). That is,

genotypes fall much closer to expected proportions in treated groups. In acephate-treated larvae of all ages, the presence of the silent allele appears to have been nearly eliminated. In carbaryl-treated larval groups, fewer silent alleles occur in 6th instars than other instars.

When frequencies of the two active alleles (EST2 allozymes 3 and 4) were compared over larval development in carbaryl and acephate untreated control groups (Table 3), insignificant differences were seen between instars. For example, in the carbaryl control group, frequencies of allozymes 3 and 4 stay at about .95 and .05, respectively, from instar 2 to instar 6.

In treated groups, however, there appears to be an increase in frequency of allozyme 3 and a decrease in frequency of allozyme 4 as development proceeds (Table 3). For example, in carbaryl-treated larvae, the frequency of allozyme 3 goes from .87 in 2nd instars to .99 in 6th instars. In correspondence, allozyme 4 goes from .13 to .01. Figure 3 illustrates more clearly the changes which occur over development in the frequencies of allozyme 3 at EST2. The differences between treated and untreated insects within each instar in both the carbaryl and acephate tests show a consistent trend. That is, treated instars 2 and 3 have a significantly lower frequency of allozyme 3 than untreated insects of the same age. In later instars, there appears to be a higher proportion of allozyme 3 than in untreated groups at this stage.

Table 1. Comparative response of C. occidentalis instars 2-6 to acephate (Orthene) and carbaryl (Sevin) sprays.

| Instar | N | No. controls | C ± S.E. | BETA ± S.E. | $LD_{50}$ [a/] | 95%CL | $LD_{90}$ | 95%CL | Log L |
|---|---|---|---|---|---|---|---|---|---|
| | | | | A. ACEPHATE | | | | | |
| 2 | 400 | 40 | 0.144 ± 0.24 | 4.34 ± 0.63 | 51.7 | 42.8-60.4 | 102.0 | 84.5-137.9 | -193.6 |
| 3 | 400 | 40 | 0.121 ± 0.028 | 3.54 ± 0.63 | 52.6 | 41.6-62.0 | 120.9 | 98.0-175.9 | -195.3 |
| 4 | 400 | 50 | 0.058 ± 0.018 | 3.40 ± 0.42 | 50.1 | 42.7-57.9 | 119.1 | 97.6-159.9 | -167.3 |
| 5 | 400 | 50 | 0 | 3.09 ± 0.27 | 44.2 | 36.2-55.0 | 114.9 | 86.0-180.6 | -131.7 |
| 6 | 320 | 50 | 0 | 3.14 ± 0.29 | 47.9 | 39.4-59.5 | 122.9 | 92.1-194.8 | -127.5 |
| | | | | B. CARBARYL | | | | | |
| 2 | 400 | 50 | 0.104 ± 0.049 | 1.95 ± 0.30 | 53.7 | 18.8-83.6 | 244.0 | 145.8-1178 | -241.7 |
| 3 | 400 | 50 | 0.101 ± 0.042 | 2.02 ± 0.30 | 64.8 | 48.6-80.4 | 280.4 | 207.0-4633 | -253.3 |
| 4 | 400 | 50 | 0.043 ± 0.030 | 1.73 ± 0.26 | 69.3 | 45.2-94.3 | 383.8 | 229.5-1286 | -256.8 |
| 5 | 400 | 50 | 0.081 ± 0.039 | 1.33 ± 0.26 | 70.4 | 47.6-94.6 | 651.2 | 357.3-2341 | -272.9 |
| 6 | 400 | 50 | 0 | 1.01 ± 0.22 | 62.6 | 31.6-95.0 | 1159.3 | 399.6-76700 | -265.4 |

[a/] Dosage expressed as g/ha.

Table 2. Hardy-Weinberg chi-square values for western spruce budworm instars 2-6, treated and untreated groups (carbaryl and acephate). High values of chi-square means that a significant (95% or *) or a highly significant (99% or **) deficiency of heterozygotes (genotype 3-4) and an excess of homozygotes (genotypes 3-3 and 4-4) occurs. That is, the higher the chi-square value, the greater proportion of the silent allele there is in the group.

A. Carbaryl

| Instar | Untreated | Treated |
|---|---|---|
| 2 | 18.18** | 6.71** |
| 3 | 14.29** | 5.84* |
| 4 | 12.58** | 9.28** |
| 5 | 12.81** | 6.11* |
| 6 | 12.88** | 0.01 |

B. Acephate

| Instar | Untreated | Treated |
|---|---|---|
| 2 | 8.74** | 0.03 |
| 3 | 10.97** | 1.61 |
| 4 | 11.30** | 0.00 |
| 5 | 27.25** | 0.00 |
| 6 | 19.76** | 0.01 |

Table 3. Frequencies of EST2 allozymes 3 and 4 in untreated and treated (carbaryl and acephate) western spruce budworms instars 2-6.

| Allozyme | Instar 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| A. Carbaryl | | | | | |
| UNTREATED: | | | | | |
| p(3) | .95 | .96 | .95 | .94 | .96 |
| p(4) | .05 | .04 | .05 | .06 | .04 |
| N | 351 | 380 | 423 | 407 | 379 |
| TREATED: (dosages 10-25 pooled by instar) | | | | | |
| p(3) | .87 | .86 | .92 | .92 | .99 |
| p(4) | .13 | .14 | .08 | .08 | .01 |
| N | 36 | 37 | 73 | 64 | 98 |
| B. Orthene | | | | | |
| UNTREATED: | | | | | |
| P(3) | .97 | .96 | .98 | .94 | .95 |
| p(4) | .03 | .04 | .02 | .06 | .05 |
| N | 417 | 425 | 509 | 475 | 465 |
| TREATED: (dosages 6-25 pooled by instar) | | | | | |
| p(3) | .90 | .91 | .99 | 1.0 | .98 |
| p(4) | .10 | .09 | .01 | 0 | .02 |
| N | 31 | 37 | 66 | 40 | 71 |

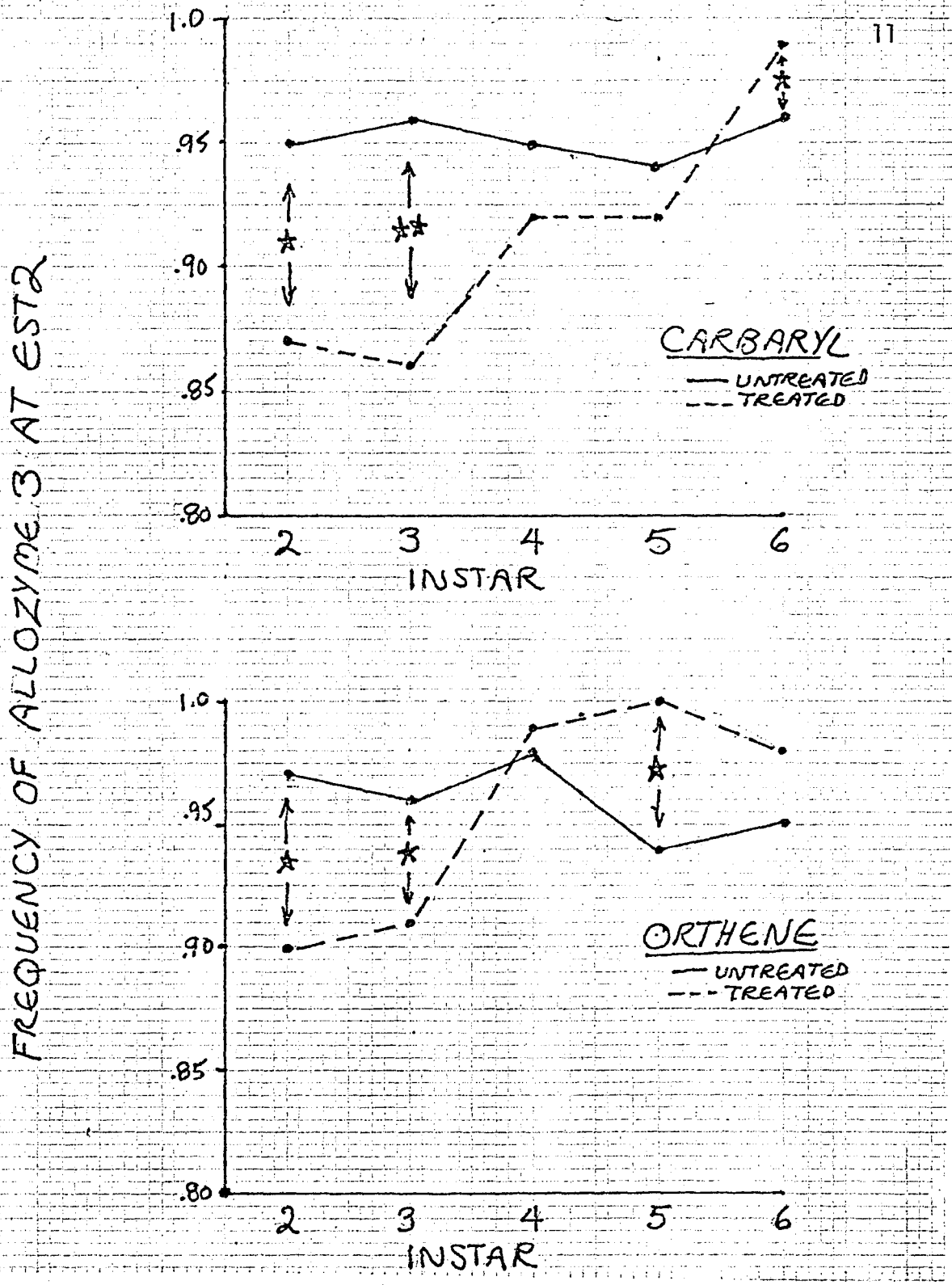


Figure 1. Comparison of the frequency of EST2 allozyme 3 in treated and untreated western spruce budworm instars 2-6. Significant (95%) and highly significant (99%) differences in frequency are indicated by * and **, respectively. Insecticide treatment appears to be associated with a decrease in frequency of allozyme 3 in early instars and an increase in allozyme 3 in later instars.

## 5. CONCLUSIONS

In general, tolerance to carbaryl increases through larval development in the western spruce budworm. These observations emphasize the necessity for careful timing of field spray operations in relation to population structure of this species.

Shifts observed in the frequency of EST2 allozyme 3 in treated 6th instars are consistent with earlier observations (Robertson and Stock 1980) that acephate-tolerant groups of 6th instars can be characterized by high proportions of this allozyme. This work shows that, in later instars (5th-6th), both carbaryl and acephate appear to select for insects with allozyme 3, though this effect does not occur in younger instars and, in fact, may be quite the reverse in early development.

Genetic data presented here also suggest that the frequency of the silent allele in western spruce budworm populations diminishes as a direct result of carbaryl and acephate treatment. Insects bearing one or two doses of the silent allele (i.e., heterozygous or homozygous for this allele) appear to have a lower tolerance to these two chemicals. Insects bearing active EST2 alleles might be better able, because of quantitative differences in the amount of esterase present, to overcome the toxic effects of these chemicals. This conclusion is in agreement with what is known about the role of esterases and the mode of action of organophosphorous (e.g., Orthene) and carbamate (e.g., Sevin) insecticides. Selection against the silent allele appears uniformly over all instars when acephate is used. For carbaryl, the greatest shift in favor of active EST2 alleles (or against the silent EST2 allele) occurs in 6th instars. In earlier work (Robertson and Stock 1980), we determined that the frequency of the silent allele was approximately one-third less in the laboratory C. occidentalis colony than in C. occidentalis from a field popu-

lation from McCall, Idaho. Frequencies of active alleles were correspondingly higher in the laboratory colony. The laboratory insects were much more resistant to acephate treatment. Thus, it appears that the frequency of the silent allele might indicate levels of tolerance in budworm populations, in addition to frequencies of EST2 allozyme 3.

To establish the practical utility of using genetic markers to detect insecticide tolerance, an investigation comparing western spruce budworm responses to carbaryl in relation to the frequencies of the silent allele and allozyme 3 at EST2 should be undertaken. Integration of this data with data for population instar frequencies and tolerance characteristics would provide a useful decision tool to determine whether carbaryl is an appropriate chemical to apply, or whether innate tolerance to this chemical would preclude its use.

6. WORK REMAINING - Preparation of manuscript.

7. COOPERATION _ This work was a cooperative effort between the Department of Forest Resources, University of Idaho, and the Pacific Southwest Forest and Range Experiment Station, Berkeley.

8. PROBLEMS ENCOUNTERED - None. Suggestions for follow-up research are given in the last paragraph of section 5. Conclusions.

9. MANUSCRIPTS PLANNED - A manuscript entitled "Effects of Insecticides on Esterase Isozymes of Western Spruce Budworm during Larval Development" is in preparation for submission to Developmental Biology.

Appendix - LITERATURE CITED

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